

WHAT IS CLAIMED IS:

1. An oligonucleotide or assembly of oligonucleotides useful in detecting a presence or an absence of a target nucleic acid sequence in a sample, the oligonucleotide or assembly of oligonucleotides comprising:

- (a) a first region and a second region, at least a portion of said first region and at least a portion of said second region each being capable of hybridizing under predetermined hybridization conditions with the target nucleic acid sequence; and
- (b) a third region and a fourth region, said third region and said fourth region being linked to said first region and said second region, respectively, a first portion and a second portion of said oligonucleotide or assembly of oligonucleotides being capable of forming a first duplex structure therebetween under said predetermined hybridization conditions;

said first, second, third and fourth regions of the oligonucleotide or assembly of oligonucleotides being selected such that upon hybridization under said predetermined hybridization conditions of said first region and said second region with said target nucleic acid sequence, said first duplex structure dissociates and a portion of said third region and a portion of said fourth region form a second duplex structure therebetween, said second duplex structure including a nucleic acid cleaving agent recognition sequence which is absent from said first duplex structure and which, when cleaved, indicates hybridization of the oligonucleotide or assembly of oligonucleotides to the target nucleic acid sequence and therefore indicates the presence of the target nucleic acid in the sample.

2. The oligonucleotide or assembly of oligonucleotides of claim 1, wherein said first portion and said second portion of said oligonucleotide or assembly of oligonucleotides being capable of forming said first duplex structure therebetween under said predetermined hybridization conditions are derived from said third and fourth regions, respectively.

3. The oligonucleotide or assembly of oligonucleotides of claim 1, wherein said first, second, third and fourth regions of the oligonucleotide or assembly of oligonucleotides are further selected such that following cleavage of said nucleic acid cleaving agent recognition sequence, said first and second

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regions dissociate from the target nucleic acid sequence, thereby enabling recycling of the target nucleic acid sequence.

4. The oligonucleotide or assembly of oligonucleotides of claim 1, further comprising at least one detection moiety linked to the oligonucleotide or assembly of oligonucleotides in a manner so as to enable detection of cleavage of said nucleic acid cleaving agent recognition sequence.

5. The oligonucleotide or the assembly of oligonucleotides of claim 4, wherein said at least one detection moiety is selected from the group consisting of at least one directly detectable detection moiety and at least one indirectly detectable detection moiety.

6. The oligonucleotide or the assembly of oligonucleotides of claim 5, wherein said at least one directly detectable detection moiety is selected from the group consisting of a fluorescent moiety and a radioactive moiety and further wherein said at least one indirectly detectable detection moiety is selected from the group consisting of at least one member of a binding pair and at least one member of a chemically interacting pair.

7. The oligonucleotide or the assembly of oligonucleotides of claim 6, wherein said at least one member of said binding pair is selected from the group consisting of an antibody, an antigen, an epitope, a ligand, a receptor, biotin, avidin, streptavidin, an ion and a chelator, and further wherein said at least one member of said chemically interacting pair is selected from the group consisting of an enzyme, a catalyst and a substrate.

8. The oligonucleotide or the assembly of oligonucleotides of claim 5, wherein said at least one detection moiety includes a resonantly interacting pair of detection moieties, said resonantly interacting pair of detection moieties including a first detection moiety and a second detection moiety, whereas said first detection moiety and said second detection moiety are selected such that at least one of said first detection moiety and said second detection moiety is capable of producing a detectable signal when in a non-interacting distance from the other detection moiety, so that a signal is produceable by one of said first detection moiety and said second detection moiety upon cleavage of said nucleic acid recognition sequence.

9. The oligonucleotide or the assembly of oligonucleotides of claim 8, wherein said first and second detection moieties form a fluorescence resonance energy transfer pair.

10. The oligonucleotide or the assembly of oligonucleotides of claim 8, wherein said first detection moiety is a fluorescer and further wherein said second detection moiety is a quencher of said fluorescer.

11. The oligonucleotide or the assembly of oligonucleotides of claim 10, wherein said fluorescer is EDANS and further wherein said quencher is DABSYL.

12. The oligonucleotide or assembly of oligonucleotides of claim 1, comprising a single oligonucleotide.

13. The oligonucleotide or assembly of oligonucleotides of claim 1, comprising a pair of oligonucleotides.

14. A method of detecting a presence or an absence of a target nucleic acid sequence in a sample, the method comprising the steps of:

- (a) contacting the sample with an oligonucleotide or assembly of oligonucleotides under predetermined hybridization conditions so as to form a reaction mixture, said oligonucleotide or assembly of oligonucleotides including:
 - (i) a first region and a second region, at least a portion of said first region and at least a portion of said second region each being capable of hybridizing with the target nucleic acid sequence; and
 - (ii) a third region and a fourth region, said third region and said fourth region being linked to said first region and said second region, respectively, a first portion and a second portion of said oligonucleotide or assembly of oligonucleotides being capable of forming a first duplex structure therebetween under said predetermined hybridization conditions;
 said first, second, third and fourth regions of the oligonucleotide or assembly of oligonucleotides being selected such that upon hybridization under said predetermined hybridization

(b) adding a nucleic acid cleaving agent to said reaction mixture, such that, if the target nucleic acid sequence is present in the sample, said nucleic acid cleaving agent recognition sequence is formed and cleaved by said cleaving agent; and

(c) monitoring cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent;

15. The method of claim 14, wherein said first portion and said second portion of said oligonucleotide or assembly of oligonucleotides being capable of forming said first duplex structure therebetween under said predetermined hybridization conditions are derived from said third and forth regions, respectively.

17. The method of claim 14, wherein said oligonucleotide or the assembly of oligonucleotides includes at least one detection moiety linked thereto in a manner so as to enable detection of cleavage of said nucleic acid cleaving agent recognition sequence.

18. The method of claim 17, wherein said at least one detection moiety is selected from the group consisting of at least one directly detectable detection moiety and at least one indirectly detectable detection moiety.

19. The method of claim 18, wherein said at least one directly detectable detection moiety is selected from the group consisting of a fluorescent moiety and a radioactive moiety and further wherein said at least one indirectly detectable detection moiety is selected from the group consisting of at least one member of a binding pair and at least one member of a chemically interacting pair.

20. The method of claim 19, wherein said at least one member of said binding pair is selected from the group consisting of an antibody, an antigen, an epitope, a ligand, a receptor, biotin, avidin, streptavidin, an ion and a chelator, and further wherein said at least one member of said chemically interacting pair is selected from the group consisting of an enzyme, a catalyst and a substrate.

21. The method of claim 18, wherein said at least one detection moiety includes a resonantly interacting pair of detection moieties, said resonantly interacting pair of detection moieties including a first detection moiety and a second detection moiety, whereas said first detection moiety and said second detection moiety are selected such that at least one of said first detection moiety and said second detection moiety is capable of producing a detectable signal when in a non-interacting distance from the other detection moiety, so that a signal is produceable by one of said first detection moiety and said second detection moiety upon cleavage of said nucleic acid recognition sequence.

22. The method of claim 21, wherein said first and second detection moieties form a fluorescence resonance energy transfer pair.

23. The method of claim 21, wherein said first detection moiety is a fluorescer and further wherein said second detection moiety is a quencher of said fluorescer.

24. The method of claim 23, wherein said fluorescer is EDANS and further wherein said quencher is DABSYL.

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25. The method of claim 14, wherein the oligonucleotide or assembly of oligonucleotides comprising a single oligonucleotide.

26. The method of claim 14, wherein the oligonucleotide or assembly of oligonucleotides comprising a pair of oligonucleotides.

27. An oligonucleotide system useful for detecting a presence or an absence of a target nucleic acid sequence in a sample comprising at least a first oligonucleotide and a second oligonucleotide, each of said first oligonucleotide and said second oligonucleotide including a first region being capable of hybridizing with the target nucleic acid sequence under predetermined hybridization conditions, each of said first oligonucleotide and said second oligonucleotide further including a second region, wherein upon hybridization, at least a portion of said second regions of said first oligonucleotide and said second oligonucleotide form a duplex structure including a nucleic acid cleaving agent recognition sequence, said second regions of said first oligonucleotide and said second oligonucleotide being selected such that in a presence of a nucleic acid cleaving agent recognizing said nucleic acid cleaving agent recognition sequence, only said first oligonucleotide is cleavable by said nucleic acid cleaving agent.

28. The oligonucleotide system of claim 27, wherein said first and second regions of said first and second oligonucleotides are selected such that upon cleavage of said first oligonucleotide, said first region of said first oligonucleotide dissociates from the target nucleic acid sequence.

29. The oligonucleotide system of claim 28, wherein said first region of said second oligonucleotide is selected such that under said predetermined hybridization conditions and following dissociation of said first oligonucleotide, said first region of said second oligonucleotide remains hybridized to the target nucleic acid sequence, thereby allowing recycling of the target nucleic acid sequence with respect to said first oligonucleotide.

30. The oligonucleotide system of claim 27, wherein at least one nucleotide or internucleotidic bond of said second oligonucleotide which forms a part of said nucleic acid cleaving agent recognition sequence is a modified or analogous nucleotide or internucleotidic bond, selected so as to prevent cleavage of said second oligonucleotide by said nucleic acid cleaving agent.

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32. The oligonucleotide system of claim 27, wherein said second regions of said first and second oligonucleotides are selected such that said nucleic acid cleaving agent recognition sequence is characterized by a nick replacing an internucleotidic bond cleavable by said nucleic acid cleaving agent.

33. The oligonucleotide system of claim 27, further comprising at least one detection moiety linked to the oligonucleotide system in a manner so as to enable detection of cleavage of said nucleic acid cleaving agent recognition sequence.

34. The oligonucleotide system of claim 33, wherein said at least one detection moiety is selected from the group consisting of at least one directly detectable detection moiety and at least one indirectly detectable detection moiety.

35. The oligonucleotide system of claim 34, wherein said at least one directly detectable detection moiety is selected from the group consisting of a fluorescent moiety and a radioactive moiety and further wherein said at least one indirectly detectable detection moiety is selected from the group consisting of at least one member of a binding pair and at least one member of a chemically interacting pair.

36. The oligonucleotide system of claim 35, wherein said at least one member of said binding pair is selected from the group consisting of an antibody, an antigen, an epitope, a ligand, a receptor, biotin, avidin, streptavidin, an ion and a chelator, and further wherein said at least one member of said chemically interacting pair is selected from the group consisting of an enzyme, a catalyst and a substrate.

37. The oligonucleotide system of claim 34, wherein said at least one detection moiety includes a resonantly interacting pair of detection moieties, said resonantly interacting pair of detection moieties including a first detection moiety and a second detection moiety, whereas said first detection moiety and

said second detection moiety are selected such that at least one of said first detection moiety and said second detection moiety is capable of producing a detectable signal when in a non-interacting distance from the other detection moiety, so that a signal is produceable by one of said first detection moiety and said second detection moiety upon cleavage of said nucleic acid recognition sequence.

38. The oligonucleotide system of claim 37, wherein said first and second detection moieties form a fluorescence resonance energy transfer pair.

39. The oligonucleotide system of claim 37, wherein said first detection moiety is a fluorescer and further wherein said second detection moiety is a quencher of said fluorescer.

40. The oligonucleotide system of claim 39, wherein said fluorescer is EDANS and further wherein said quencher is DABSYL.

41. A method of detecting a presence or an absence of a target nucleic acid sequence in a sample, the method comprising the steps of:

- (a) contacting the sample with an oligonucleotide system under hybridization conditions so as to form a reaction mixture, said oligonucleotide system including at least a first oligonucleotide and a second oligonucleotide, each of said first oligonucleotide and said second oligonucleotide including a first region being capable of hybridizing under predetermined hybridization conditions with the target nucleic acid sequence, each of said first oligonucleotide and said second oligonucleotide further including a second region, wherein upon hybridization under said predetermined hybridization conditions, at least a portion of said second regions of said first oligonucleotide and said second oligonucleotide form a duplex structure including a nucleic acid cleaving agent recognition sequence, said second regions of said first oligonucleotide and said second oligonucleotide being selected such that in a presence of a nucleic acid cleaving agent recognizing said nucleic acid cleaving agent recognition sequence, only said first oligonucleotide is cleavable by said nucleic acid cleaving agent;

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- (b) adding said nucleic acid cleaving agent to said reaction mixture, such that, if the target nucleic acid sequence is present in the sample, said nucleic acid cleaving agent recognition sequence is cleaved by said nucleic acid cleaving agent; and
- (c) monitoring cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent;

wherein cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent indicates hybridization of the oligonucleotide system to the target nucleic acid sequence and therefore the presence of the target nucleic acid in the sample.

42. The method of claim 41, wherein detecting a presence or an absence of said cleavage is effected by monitoring the presence or absence of specific cleavage products.

43. The method of claim 41, wherein said first and second regions of said first and second oligonucleotides are selected such that upon cleavage of said first oligonucleotide, said first region of said first oligonucleotide dissociates from the target nucleic acid sequence.

44. The method of claim 43, wherein said first region of said second oligonucleotide is selected such that under said predetermined hybridization conditions and following dissociation of said first oligonucleotide, said first region of said second oligonucleotide remains hybridized to the target nucleic acid sequence, thereby allowing recycling of the target nucleic acid sequence with respect to said first oligonucleotide.

45. The method of claim 41, wherein at least one nucleotide or internucleotidic bond of said second oligonucleotide which forms a part of said nucleic acid cleaving agent recognition sequence is a modified or analogous nucleotide or internucleotidic bond, selected so as to prevent cleavage of said second oligonucleotide by said nucleic acid cleaving agent.

46. The method of claim 41, wherein said duplex structure is formed in part by self annealing of a portion of said second region of said first oligonucleotide.

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47. The method of claim 41, wherein said second regions of said first and second oligonucleotides are selected such that said nucleic acid cleaving agent recognition sequence is characterized by a nick replacing an internucleotidic bond cleavable by said nucleic acid cleaving agent.

48. The method of claim 41, wherein said oligonucleotide sequence further comprising at least one detection moiety linked to the oligonucleotide system in a manner so as to enable detection of cleavage of said nucleic acid cleaving agent recognition sequence.

49. The method of claim 48, wherein said at least one detection moiety is selected from the group consisting of at least one directly detectable detection moiety and at least one indirectly detectable detection moiety.

50. The method of claim 49, wherein said at least one directly detectable detection moiety is selected from the group consisting of a fluorescent moiety and a radioactive moiety and further wherein said at least one indirectly detectable detection moiety is selected from the group consisting of at least one member of a binding pair and at least one member of a chemically interacting pair.

51. The method of claim 50, wherein said at least one member of said binding pair is selected from the group consisting of an antibody, an antigen, an epitope, a ligand, a receptor, biotin, avidin, streptavidin, an ion and a chelator, and further wherein said at least one member of said chemically interacting pair is selected from the group consisting of an enzyme, a catalyst and a substrate.

52. The method of claim 49, wherein said at least one detection moiety includes a resonantly interacting pair of detection moieties, said resonantly interacting pair of detection moieties including a first detection moiety and a second detection moiety, whereas said first detection moiety and said second detection moiety are selected such that at least one of said first detection moiety and said second detection moiety is capable of producing a detectable signal when in a non-interacting distance from the other detection moiety, so that a signal is produceable by one of said first detection moiety and said second detection moiety upon cleavage of said nucleic acid recognition sequence.

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54. The method of claim 52, wherein said first detection moiety is a fluorescer and further wherein said second detection moiety is a quencher of said fluorescer.

56. An oligonucleotide system useful for detecting a presence or an absence of a target nucleic acid sequence in a sample comprising at least a first oligonucleotide and a second oligonucleotide, each of said first oligonucleotide and said second oligonucleotide including a first region being complementary or substantially complementary to the target nucleic acid sequence, each of said first oligonucleotide and said second oligonucleotide further including a second region, said second regions of said first and second oligonucleotides being complementary or substantially complementary and being selected such that upon annealing therebetween said second regions form a duplex structure including a nucleic acid cleaving agent recognition sequence, wherein under predetermined hybridization conditions said first region of said first oligonucleotide is stably hybridizable with said target nucleic acid sequence only if said first region of said second oligonucleotide is stably hybridizable with said nucleic acid target sequence.

57. The oligonucleotide system of claim 56, wherein under said predetermined hybridization conditions said second regions of said first and second oligonucleotides are substantially non-hybridizable with one another per se.

58. The oligonucleotide system of claim 56, wherein said second regions of said first oligonucleotide and said second oligonucleotide are selected such that in a presence of a nucleic acid cleaving agent recognizing said nucleic acid cleaving agent recognition sequence, only said first oligonucleotide is cleavable by said nucleic acid cleaving agent.

59. The oligonucleotide system of claim ~~58~~, wherein said first and second regions of said first and second oligonucleotides are selected such that

under said predetermined hybridization conditions and upon cleavage of said first oligonucleotide, said first region of said first oligonucleotide dissociates from the target nucleic acid sequence.

60. The oligonucleotide system of claim 58, wherein at least one nucleotide or internucleotidic bond of said second oligonucleotide which forms a part of said nucleic acid cleaving agent recognition sequence is a modified or analogous nucleotide or internucleotidic bond, selected so as to prevent cleavage of said second oligonucleotide by said nucleic acid cleaving agent.

61. The oligonucleotide system of claim 58, wherein said duplex structure is formed in part by self annealing of a portion of said second region of said first oligonucleotide.

62. The oligonucleotide system of claim 58, wherein said second regions of said first and second oligonucleotides are selected such that said nucleic acid cleaving agent recognition sequence is characterized by a nick replacing an internucleotidic bond cleavable by said nucleic acid cleaving agent.

63. The oligonucleotide system of claim 56, further comprising at least one detection moiety linked to the oligonucleotide system in a manner so as to enable detection of cleavage of said nucleic acid cleaving agent recognition sequence.

64. The oligonucleotide system of claim 63, wherein said at least one detection moiety is selected from the group consisting of at least one directly detectable detection moiety and at least one indirectly detectable detection moiety.

65. The oligonucleotide system of claim 64, wherein said at least one directly detectable detection moiety is selected from the group consisting of a fluorescent moiety and a radioactive moiety and further wherein said at least one indirectly detectable detection moiety is selected from the group consisting of at least one member of a binding pair and at least one member of a chemically interacting pair.

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(a) contacting the sample with an oligonucleotide system so as to form a reaction mixture, said oligonucleotide system including at least a first oligonucleotide and a second oligonucleotide, each of said first oligonucleotide and said second oligonucleotide including a first region being complementary or substantially complementary to the target nucleic acid sequence, each of said first oligonucleotide and said second oligonucleotide further including a second region, said second regions of said first and

second oligonucleotides being complementary or substantially complementary and being selected such that upon annealing therebetween said second regions form a duplex structure including a nucleic acid cleaving agent recognition sequence, wherein under said predetermined hybridization conditions said first region of said first oligonucleotide is stably hybridizable with said target nucleic acid sequence only if said first region of said second oligonucleotide is stably hybridizable with said nucleic acid target sequence;

- (b) adding a nucleic acid cleaving agent to said reaction mixture, such that, if the target nucleic acid sequence is present in the sample, said nucleic acid cleaving agent recognition sequence is cleaved by said nucleic acid cleaving agent; and
- (c) monitoring cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent;

wherein cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent indicates hybridization of the oligonucleotide system to the target nucleic acid sequence and therefore the presence of the target nucleic acid in the sample.

72. The method of claim 71, wherein detecting a presence or an absence of said cleavage is effected by monitoring the presence or absence of specific cleavage products.

73. The method of claim 71, wherein said first and second regions of said first and second oligonucleotides are selected such that under said predetermined hybridization conditions and upon cleavage of said first oligonucleotide, said first region of said first oligonucleotide dissociates from the target nucleic acid sequence.

74. The method of claim 71, wherein at least one nucleotide or internucleotidic bond of said second oligonucleotide which forms a part of said nucleic acid cleaving agent recognition sequence is a modified or analogous nucleotide or internucleotidic bond, selected so as to prevent cleavage of said second oligonucleotide by said nucleic acid cleaving agent.

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75. The method of claim 71, wherein said duplex structure is formed in part by self annealing of a portion of said second region of said first oligonucleotide.

76. The method of claim 71, wherein said second regions of said first and second oligonucleotides are selected such that said nucleic acid cleaving agent recognition sequence is characterized by a nick replacing an internucleotidic bond cleavable by said nucleic acid cleaving agent.

77. The method of claim 71, wherein said oligonucleotide sequence further comprising at least one detection moiety linked to the oligonucleotide system in a manner so as to enable detection of cleavage of said nucleic acid cleaving agent recognition sequence.

78. The method of claim 77, wherein said at least one detection moiety is selected from the group consisting of at least one directly detectable detection moiety and at least one indirectly detectable detection moiety.

79. The method of claim 78, wherein said at least one directly detectable detection moiety is selected from the group consisting of a fluorescent moiety and a radioactive moiety and further wherein said at least one indirectly detectable detection moiety is selected from the group consisting of at least one member of a binding pair and at least one member of a chemically interacting pair.

80. The method of claim 79, wherein said at least one member of said binding pair is selected from the group consisting of an antibody, an antigen, an epitope, a ligand, a receptor, biotin, avidin, streptavidin, an ion and a chelator, and further wherein said at least one member of said chemically interacting pair is selected from the group consisting of an enzyme, a catalyst and a substrate.

81. The method of claim 78, wherein said at least one detection moiety includes a resonantly interacting pair of detection moieties, said resonantly interacting pair of detection moieties including a first detection moiety and a second detection moiety, whereas said first detection moiety and said second detection moiety are selected such that at least one of said first detection moiety and said second detection moiety is capable of producing a detectable signal when in a non-interacting distance from the other detection

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moiety, so that a signal is produceable by one of said first detection moiety and said second detection moiety upon cleavage of said nucleic acid recognition sequence.

82. The method of claim 81, wherein said first and second detection moieties form a fluorescence resonance energy transfer pair.

83. The method of claim 81, wherein said first detection moiety is a fluorescer and further wherein said second detection moiety is a quencher of said fluorescer.

84. The method of claim 83, wherein said fluorescer is EDANS and further wherein said quencher is DABSYL.

85. An oligonucleotide system useful for detecting a presence or an absence of a target nucleic acid sequence in a sample comprising at least a first oligonucleotide and a second oligonucleotide, each of said first oligonucleotide and said second oligonucleotide including a first region being complementary or substantially complementary to the target nucleic acid sequence, each of said first oligonucleotide and said second oligonucleotide further including a second region, said second regions of said first and second oligonucleotides being complementary or substantially complementary and being selected such that upon annealing therebetween said second regions form a duplex structure including a nucleic acid cleaving agent-recognition sequence, wherein under predetermined hybridization conditions said first regions of said first oligonucleotide and said second oligonucleotide are stably hybridizable with said target nucleic acid sequence, and said second regions of said first oligonucleotide and said second oligonucleotide are stably hybridizable therebetween only when said first oligonucleotide, said second oligonucleotide and said target nucleic acid sequence are co-annealed.

86. A method of detecting a presence or an absence of a target nucleic acid sequence in a sample, the method comprising the steps of:

- (a) contacting the sample with an oligonucleotide system so as to form a reaction mixture, said oligonucleotide system including at least a first oligonucleotide and a second oligonucleotide, each of said first oligonucleotide and said second oligonucleotide including a first region being complementary or substantially

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complementary to the target nucleic acid sequence, each of said first oligonucleotide and said second oligonucleotide further including a second region, said second regions of said first and second oligonucleotides being complementary or substantially complementary and being selected such that upon annealing therebetween said second regions form a duplex structure including a nucleic acid cleaving agent recognition sequence, wherein under predetermined hybridization conditions said first regions of said first oligonucleotide and said second oligonucleotide are stably hybridizable with said target nucleic acid sequence, and said second regions of said first oligonucleotide and said second oligonucleotide are stably hybridizable therebetween only when said first oligonucleotide, said second oligonucleotide and said target nucleic acid sequence are co-annealed;

- (b) adding a nucleic acid cleaving agent to said reaction mixture, such that, if the target nucleic acid sequence is present in the sample, said nucleic acid cleaving agent recognition sequence is cleaved by said nucleic acid cleaving agent; and
- (c) monitoring cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent;

wherein cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent indicates hybridization of the oligonucleotide system to the target nucleic acid sequence and therefore the presence of the target nucleic acid in the sample.

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